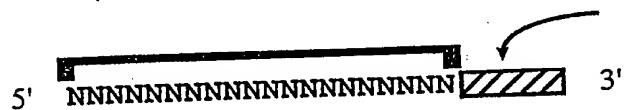


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APPROVED	J.G. FIG.
BY	CLASS SUBCLASS
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**A) Design and Synthesis of Nucleic Acids (oligonucleotides):**  
i) *Substrate Nucleic Acid*

## 5' Specific Sequence Domain (TARGET BINDING DOMAIN)



**Universal TEMPLATE  
HYBRIDIZATION DOMAIN  
(same for all probes)**

ii) *Template Nucleic Acid*

The diagram illustrates the hybridization mechanism. At the top, a horizontal line represents the **Extension Template (SIGNAL TEMPLATE DOMAIN)**. Below it, another horizontal line represents the **SUBSTRATE**. A hatched box labeled **HYBRIDIZATION DOMAIN** is positioned between them. An arrow points from the Extension Template to the Substrate, indicating the complementary nature of the domains.

B) Anneal Substrate + Template Nucleic Acids in Buffer (10 minutes)



C) Labeling / Extension Reaction (1 hour)  
Add DNA Polymerase +  $\alpha$ -<sup>32</sup>P-dATP [\*A]



## Labeled Substrate Nucleic Acid (PROBE)

D) Remove Unincorporated Label using Column Chromatography (10 minutes)  
Optional: Remove Template and more highly purify Probe using PAGE (2 hour gel + elute)

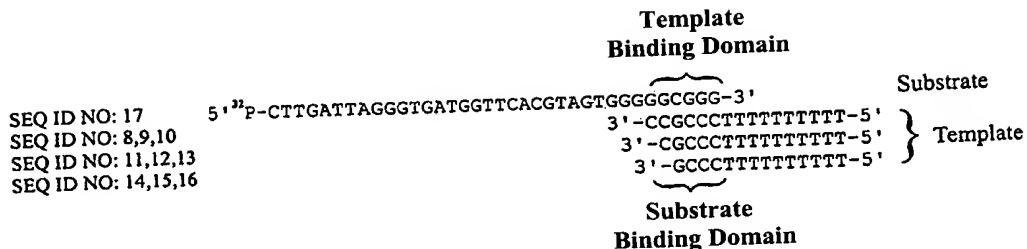


**FIG. 1**

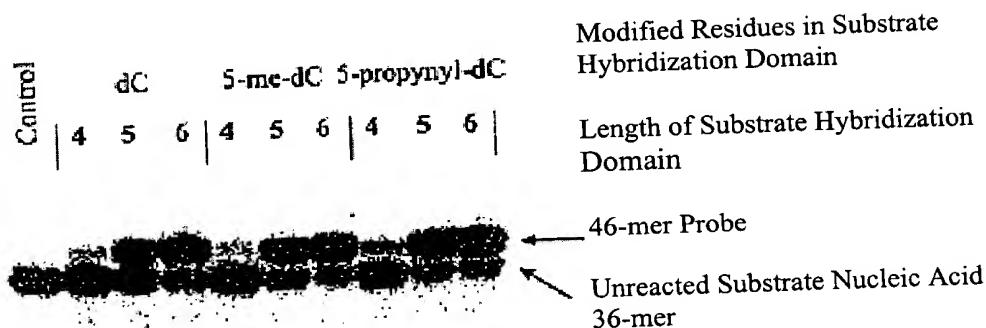
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## A) Substrate and Template Nucleic Acids



## B) PAGE analysis of reaction products

**FIG. 2**

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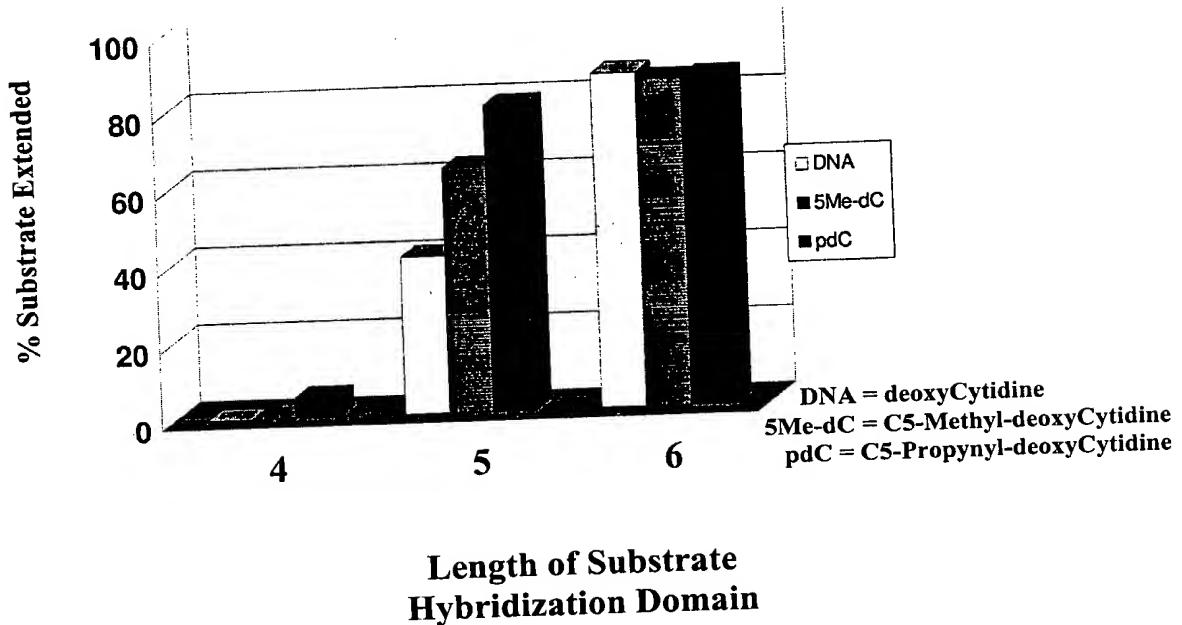


FIG. 3

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APPROVED	O.G. FIG.
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pGreen Lantern™-1 Probes

Kinase Labeled Probe



High Specific Activity Tailed Probe

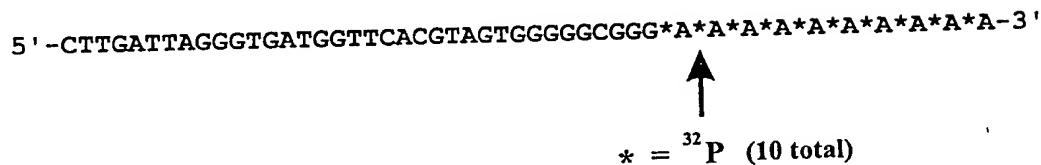


FIG. 4A

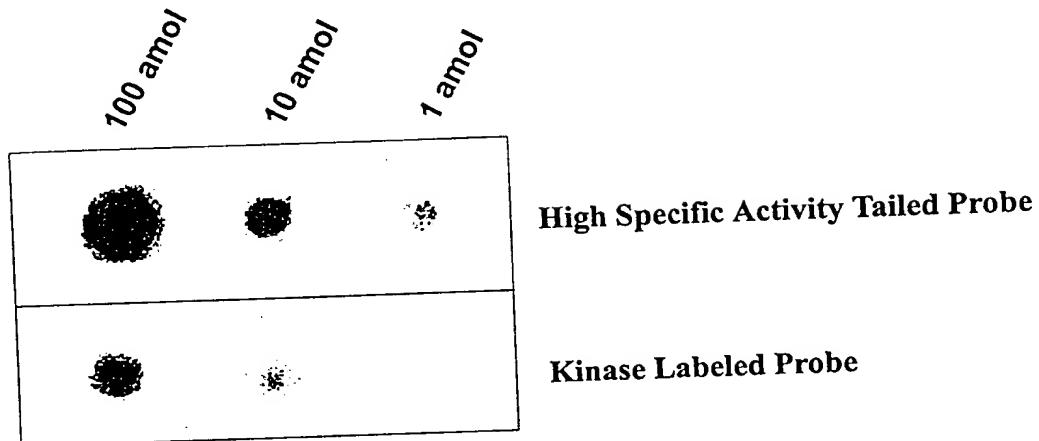
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(SHEET 5 OF 9)

APPROVED	J.G. FIG.
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**Dot Blot**

Mass of target plasmid on Membrane



**FIG. 4B**

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APPROVED	O.G. FIG.
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## β-Actin Probes

### Kinase Labeled Probe

5' -<sup>32</sup>P-GCCCAGGAAGGAAGGCTGGAAGAGTGCCTCGCGGG-3'

### High Specific Activity Tailed Probe

5' -GCCAGGAAGGAAGGCTGGAAGAGTGCCTCGCGGG\*A\*A\*A\*A\*A\*A\*A\*A-A-3'

↑  
\* = <sup>32</sup>P (10 total)

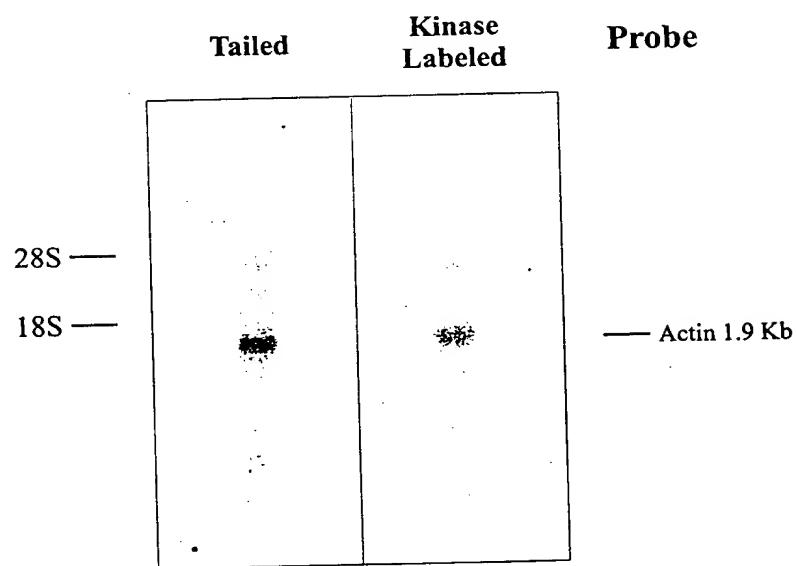
FIG. 5A

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APPROVED	O.G. FIG.
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**Northern Blot of Human Placental RNA**



**FIG. 5B**

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APPROVED	O.G. FIG.
CLASS	SUBCLASS
BY	

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## Substrate Nucleic Acids and Reaction Products

SEQ ID NO: 21 DNA Substrate Nucleic Acid

5' <sup>32</sup>P-GCCCAGGAAGGAAGGCTGGAAAGAGTGCCTCGGCCGG-3'

SEQ ID NO: 22 DNA Reaction Product

5' <sup>32</sup>P-GCCCAGGAAGGAAGGCTGGAAAGAGTGCCTCGGCCGGAAAAAAAAAA-3'

SEQ ID NO: 24 RNA Substrate Nucleic Acid

5' <sup>32</sup>P-cugggcauggaguccuguggcauccacgaaacuaccuuucaggcggg-3'

SEQ ID NO: 25 RNA Reaction Product

5' <sup>32</sup>P-cugggcauggaguccuguggcauccacgaaacuaccuuucaggcgggAAAAAAAAAA-3'

FIG. 6A

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APPROVED	O. G. FIG.
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PAGE analysis of Reaction Products

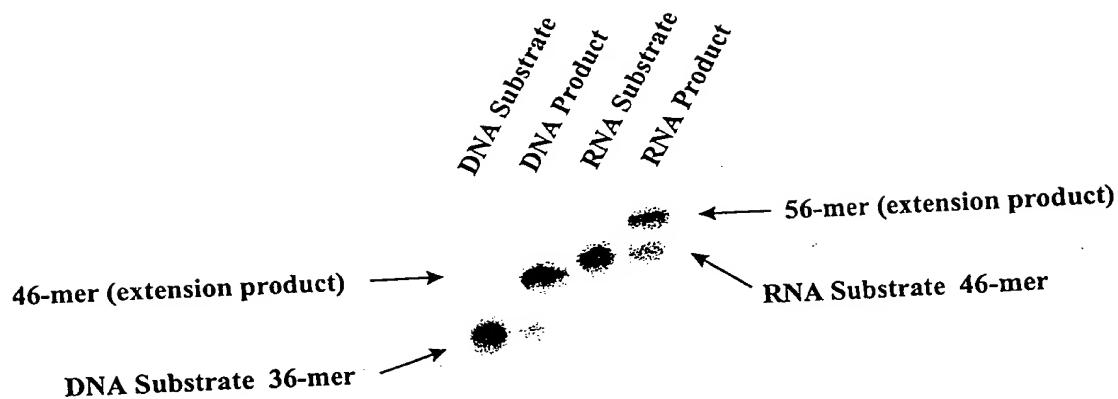


FIG. 6B